Influence of abomasal carbohydrates on subcutaneous, omental, and mesenteric adipose lipogenic and lipolytic rates in growing beef steers

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Influence of abomasal carbohydrates on subcutaneous, omental, and mesenteric adipose lipogenic and lipolytic rates in growing beef steers

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ABSTRACT: To determine the response to alteration in site and form of carbohydrate delivery to the digestive tract, in vitro rates of lipogenesis and lipolysis in mesenteric (MESA), omental (OMA), and subcutaneous (SQA) adipose depots were compared. Forty crossbred beef steers (243 ± 2 kg of BW) were fed 161 (LI) or 214 (HI) kcal of ME/(kg of $BW^{0.75}$·d) or they were fed LI and infused for 35 d into the rumen (R) or abomasum (A) with starch hydrolysate (SH) or into the abomasum with glucose (G). Jugular blood samples were collected, steers were slaughtered, and adipose depots were sampled and prepared for assessment of lipogenesis and lipolysis in vitro. Blood concentrations of glucagon were increased ($P = 0.04$) in HI-H_2O compared with LI-H_2O steers, whereas A-SH tended to increase ($P = 0.08$) circulating IGF-I relative to R-SH, and A-G tended to have elevated ($P = 0.09$) T_3 compared with A-SH. Lipolysis, as assessed by NEFA release, was unaffected by treatment. Glycerol release by the MESA and SQA was increased or tended to be increased ($P \leq 0.08$) in HI-H_2O compared with LI-H_2O steers. In A-G compared with A-SH steers, glycerol release from OMA increased ($P = 0.008$) and from SQA tended to be increased ($P = 0.08$). Acetate incorporation into total neutral lipids (TNL) increased or tended to increase with ME intake and SH infusion ($P \leq 0.09$) across all depots. Rates of acetate incorporation into fatty acids (FA) also increased or tended to be increased ($P \leq 0.1$) by SH infusion across all depots, but only that of SQA was increased with ME intake (HI-H_2O vs. LI-H_2O; $P = 0.02$). Rates of acetate incorporation into FA and TNL in MESA were increased ($P \leq 0.03$) by A-SH compared with R-SH, but site of SH infusion did not affect the rates in SQA or OMA. Glucose incorporation into TNL for MESA and SQA increased or tended to be increased ($P \leq 0.1$) by dietary and infused energy, whereas for OMA they tended to be increased ($P = 0.1$) only by SH infusion. In contrast, glucose incorporation into FA was unaffected by energy supply but tended to be increased ($P = 0.07$) by SH in MESA and tended to be greater ($P = 0.08$) for A-G than A-SH in OMA. The general across-depot pattern of acetate incorporation rate into FA and TNL was SQA > OMA > MESA, whereas, for glucose incorporation, rates across depots were equivalent. These data provide evidence that the postruminal supply of energy, specifically carbohydrate, stimulates lipogenesis from acetate and glucose and is more pronounced in abdominal depots relative to the subcutaneous depot.

Key words: steer, carbohydrate, adipose, metabolism

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INTRODUCTION

Current beef cattle feeding models have elements to reflect the biological mechanisms controlling composition of gain. Yet protein and fat accretion predictions are based on percentage mature BW and ME intake (NRC, 1996). Protein accretion rates are based on initial and mature DNA composition of the carcass, and residual energy is assumed to be deposited as fat (Oltjen et al., 1986). Thus, changing ration formulations while maintaining ME intake would not be expected to alter the composition of gain. However, shifting starch digestion from the rumen to the small intestine increases glucose use by portal-drained viscera and peripheral tissues (Seal and Reynolds, 1993), and a significant fraction of the increase in use is via oxidation (Harmon et al., 2001). In growing steers, retained tissue energy was greater for starch digested in the small intestine than that digested ruminally (McLeod et al., 2001).

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Moreover, intestinally supplied starch resulted in a disproportionate increase in the fraction of total body tissue energy being retained as fat (67%) compared with ruminally (59%) supplied starch, as determined by respiration calorimetry.

Excess glucose is stored as lipid or glucose indirectly stimulates lipogenesis in cattle as has been described in monogastrics (Girard et al., 1997). It is unclear whether this shift in composition of retained body tissue energy is a function of the energy available for tissue gain exceeding the capacity for protein deposition or if it represents a physiological bias toward fat deposition. Acetate is thought to be the primary substrate for ruminant adipose deposition, and therefore the mechanism by which glucose alters adipose metabolism is unclear.

Thus, we sought to determine the extent to which glucose is used for lipogenesis and the extent to which lipid metabolism responds to an altered site of delivery and complexity of carbohydrate, and to assess the relative capacity of fat depots to use glucose and acetate for de novo synthesis of lipid.

MATERIALS AND METHODS

Steers and Treatments

Experimental design and animal protocols were reviewed and approved by the Beltsville Area Animal Care and Use Committee.

Details of the animals, diet, infusion protocols, and design for this experiment were presented by McLeod et al. (2007). Briefly, 40 Angus crossbred steers (243 ± 2 kg of BW), fitted with ruminal and abomasal infusion catheters, were used in a randomized complete block design to test the hypothesis that adiposity is dependent on site and complexity of carbohydrate assimilation. To minimize the variation in initial BW, the experiment was conducted using 8 blocks of steers. Within each block, all treatments were represented and the steers were assigned randomly to treatment. Across and within blocks, the initiation of treatments was offset in a manner that allowed animal slaughters to be conducted at predefined intervals to facilitate tissue sample collection and subsequent in vitro incubations, while maintaining a constant 35-d treatment period. Steers received a pelleted basal diet [89.45% orchardgrass hay, 5.0% corn gluten meal, 5.0% lignosulfonate-treated soybean meal (Soypass, Ligotech US, Rothschild, WI), and 0.50% trace mineral-salt; all on a DM basis] in 12 portions daily at 2-h intervals and were allowed ad libitum access to water.

Steers were housed in individual tie stalls (1.1 × 1.8 m) in a temperature-controlled barn and were exercised for 1 h twice weekly. Treatments (Table 1) included the pelleted basal diet fed at 161 (**LI**) or 214 (**HI**) kcal of ME/(kg of $BW^{0.75}$·d), LI plus ruminal (**R-SH**) or abomasal (**A-SH**) infusion of a partial starch hydrolysate (**SH**), and LI plus abomasal infusion of glucose (**A-G**). Metabolizable energy intake of the basal diet was designed to approximate 1.5 and 2.0 times maintenance energy requirements of growing steers. The ME value of the basal diet was determined to be 2.32 Mcal/kg of DM in a previous experiment (McLeod et al., 2001). Infusions were conducted continuously over a 22-h period daily, and rates of 12.6 and 14.4 g/(kg of $BW^{0.75}$·d) were used to achieve isoenergetic infusions of SH and glucose, respectively. The total amount of infusate (5 kg/site daily) was balanced across treatments and infusion site by infusion of water. Steers were adapted to feed intake and carbohydrate infusate incrementally over the initial 6 d of the 35-d treatment period. Steers were weighed twice weekly, and the amount of feed offered and carbohydrate infused was adjusted weekly based on the average BW from the proceeding week.

Blood Sampling and Hormone Assays

On d 35, immediately before transport to the abattoir for slaughter, jugular venous blood samples (20 mL) were collected into heparinized syringes (Sarstedt Inc., Newton, NC) and immediately place on ice for transport to the laboratory. Subsequently, whole blood samples were centrifuged (1,800 × *g*, 30 min, 4°C), plasma was harvested, and aliquots were stored at –20°C until assayed for insulin, glucagon, triiodothyronine ($\mathbf{T_3}$), thyroxine ($\mathbf{T_4}$), and IGF-I. Plasma T_3 and T_4 concentrations were determined using a commercial RIA kit, as previously described by Kahl et al. (1992; Coat-A-Count Total T_3 and Total T_4, respectively, DPC, Los Angeles, CA). Plasma insulin, glucagon, and IGF-I concentrations were determined using double-antibody RIA. Antibodies and assay conditions for determination of insulin and glucagon were previously described by Reynolds et al. (1989), whereas those for IGF-I are described by Elsasser et al. (1989). Sensitivity and intraassay variation for the hormone assays were 60 pg/mL and 1.8% for T_3, 5 ng/mL and 2.2% for T_4, 36 pg/mL and 5.3% for insulin, 5 pg/mL and 5.9% for glucagon, and 9.2 ng/mL and 5.6% for IGF-I. All samples for each hormone were evaluated in a single assay.

Tissue Collection

Steers were slaughtered (0900 and 1100) at the institutional abattoir. To maintain protein activity in harvested tissues, animal slaughters were limited to 1 or 2 per day. Within each animal block, the order of animal slaughter was determined at the onset of the treatment period, such that all treatments and treatment combinations were balanced across single or multiple animal slaughter dates and animal slaughter pairs. Steers were stunned by captive bolt, exsanguinated, and eviscerated. After slaughter, adipose tissue samples (approximately 30 g) were collected from the omental (within the lesser curvature of the abomasum), mesenteric (within the sixth loop of the small intestine caudal to the pylorus), and the subcutaneous depots (under the hide at the ischium). Adipose samples were weighed

Table 1. Treatment structure and designations

Treatment	Designation	Dietary intake, Mcal of ME/(kg of $BW^{0.75}$·d)	Infusion (g/(kg of $BW^{0.75}$·d)	
			Ruminal	Abomasal
Water[1]	LI-H_2O	0.161	0[2]	0
Water	HI-H_2O	0.214	0	0
Ruminal starch	R-SH	0.161	12.6	0
Abomasal starch	A-SH	0.161	0	12.6
Abomasal glucose[3]	A-G	0.161	0	14.4

[1]The total amount of infusate (5 kg/site daily) was balanced across treatments and infusion site by infusion of water.
[2]All values of 0 represent a 5 L/d water infusion, with no starch or glucose.
[3]Glucose infusion represents an equivalent quantity of glucose infused as for the starch infusions.

and immediately placed into an oxygenated, isotonic wash buffer [Krebs-Henseleit bicarbonate buffer, plus 25 m*M* HEPES and Essentially Fatty Acid Free Bovine Serum Albumin (Sigma Chemical Co., St. Louis, MO), pH 7.4; KH-HEPES] at 37°C for transport to the laboratory.

Tissue Incubations

Adipose tissues were maintained in oxygenated KH-HEPES at 37°C until thin slices were prepared by placing a cube of adipose onto the platform of a Stadie-Riggs microtome (Thomas Scientific, Swedesboro, NJ) and slicing. Slices were weighed and 80 to 100 mg (approximately 500-μm thickness) slices were used for incubations. All adipose tissue incubations were initiated within 2 h after slaughter. All incubations were conducted with 5 replicates at 37°C in a shaking water bath (80 oscillations/min) for 120 min. The 120-min incubation time was previously determined to be sufficient for metabolite development while ensuring maintenance of linear rates of incorporation of substrate or release of fatty acids (**FA**) and glycerol (McNamara and Hillers, 1986). Incubations were initiated with the addition of an adipose slice into 2 mL of incubation media in a 25-mL Erlenmeyer flask, followed by 20 s of gassing with O_2:CO_2 (95:5, vol/vol). The basal incubation media consisted of a Krebs salts solution supplemented with 0.25 *M* BSA and 0.25 m*M* HEPES and containing 10 m*M* acetate and 5 m*M* glucose. Estimates of lipogenesis were established using the basal media labeled with U-^{14}C-glucose or 2-^{14}C-acetate (0.5 μCi/flask) to assess the rates of substrate incorporation into FA and total neutral lipids (**TNL**). After 120 min of incubation, tissue slices were removed from the flask, blotted, and placed into Doles solution (1 part heptane, 6 parts isopropanol, Sigma Chemical Co.) overnight.

Tubes containing Doles solution and slices were aggressively shaken for 20 min (SMI MultiTube Vortexer, Model 2600, Scientific Manufacturing Industries, Emeryville, CA). Water (2 mL) and heptane (3 mL) were added, and the tubes were inverted 10 times, and after separation of the phases, the lower phase was removed by aspiration and discarded. The organic phase was washed twice with 5 mL of 0.1 *M* NaOH. Subsequently, the upper phase was quantitatively removed into a fresh tube and split for analysis of the incorporation of the labeled substrate into TNL and FA. For TNL determination, an aliquot was dried under N_2 and radioactivity determined by scintillation counting. For assessment of FA incorporation, the remainder of the organic phase was dried under N_2 and subsequently saponified, extracted, dried under N_2, and counted by scintillation counting as for the TNL. For estimation of the rates of lipolysis, after a 20-min preincubation period, the basal media was removed, discarded, and replaced with a lipolytic stimulatory media containing the basal media further supplemented with 1 μ*M* norepinephrine, 1 μM epinephrine, 1 m*M* theophyline, and 1 unit of adenosine deaminase (Sigma Chemical Co.) for an additional 120 min. Subsequently, the media was harvested for analysis of glycerol (Cat. No. E0148270, Boehringer Mannheim, Mannheim, Germany) and NEFA (Waco Chemical, Dallas, TX) release. Because adipose tissue does not reutilize glycerol, glycerol release provides an indication of hormone sensitive lipase activity, whereas FA release provides an indication of net lipolysis because it includes FFA reesterification.

Statistical Analysis

Means for incubations of adipose tissue in vitro are presented as nanomoles of substrate produced or incorporated during the 120-min incubation per milligram of wet adipose tissue added to the flask, whereas the hormone data are presented as nanograms per milliliter in circulating blood. All statistical analyses were conducted using MIXED procedures (SAS Inst. Inc., Cary, NC). Data were analyzed as a randomized complete block design, with steer group as the block (Steele and Torrie, 1980). The model included treatment and group as fixed effects. Means from replicate flasks were included by animal; thus, n = 8 for each treatment at the initiation of the study. Single degree of freedom contrasts were used to test the effects of ME intake (LI-H_2O vs. HI-H_2O), starch infusion (LI-H_2O vs. R-SH and A-SH), site of starch infusion (R-SH vs. A-SH), and form of abomasally infused carbohydrate (A-SH vs. A-G). All

Table 2. Plasma concentrations of selected hormones in steers fed at low intake (LI) or high intake (HI) and infused ruminally (R) or abomasally (A) with hydrolyzed starch (SH) or glucose (G)[1]

		LI			HI		Contrast[1]			
Item	H_2O	R-SH	A-SH	A-G	H_2O	SEM[2]	LI-H_2O vs. HI	LI-H_2O vs. R-, A-SH	R-SH vs. A-SH	A-SH vs. A-G
Insulin, pg/mL	667	913	812	697	872	99.9	0.16	0.14	0.44	0.40
Glucagon, pg/mL	74	91	72	80	125	12.1	0.004	0.61	0.21	0.60
T_4, ng/mL	48.5	49.7	49.1	49.0	50.5	4.7	0.74	0.86	0.91	0.99
T_3, ng/mL	1.02	1.12	1.16	1.34	1.08	0.08	0.36	0.29	0.43	0.09
IGF-I, ng/mL	99	103	119	121	108	7.6	0.36	0.18	0.08	0.81

[1]Probability of larger *F*-statistic.
[2]n = 8, except for LI-H_2O (n = 6) and A-G (n = 7); SEM calculated using n = 6.

data are presented as least squares means ± SEM, with the SEM calculated using the least number of observations for each measured variable. Treatment effects were considered significant at $P \leq 0.05$ and as a tendency at $P \leq 0.10$.

RESULTS

One steer assigned to the LI A-G treatment did not consume all feed offered (40% refused) and, thus, was eliminated from further analysis. Additionally, 2 steers were eliminated because whole animal responses (McLeod et al., 2007) were not consistent with their treatment peers. Finally, 1 animal assigned to the LI-H_2O treatment had incubation data that were below detection and, thus, was not included in the statistical analysis.

Mean adipose tissue wet weights added to initiate the incubations were 94.0, 92.7, and 92.8 mg for the mesenteric, omental, and subcutaneous adipose depots, respectively. All incubations were initiated within 2 h following slaughter. Animal responses to administration of treatments are presented elsewhere (McLeod et al., 2007).

Plasma concentrations of insulin (Table 2) were similar across dietary treatments. Glucagon was increased ($P = 0.004$) by increased ME intake but was unaffected by carbohydrate infusion. Plasma T_4 and T_3 concentrations were largely unaffected by treatment with the exception of a tendency ($P = 0.09$) for T_3 concentrations to be greater for A-G compared with A-SH infusion. Similarly, IGF-I concentrations were unaffected by dietary ME intake or starch infusion, but there was a tendency ($P = 0.08$) for IGF-I concentrations to be greater for A-SH vs. R-SH, with no differences between A-G and A-SH.

Acetate incorporation into TNL (Figure 1) increased ($P = 0.02$; subcutaneous adipose) or tended to increase (mesenteric, $P = 0.09$; omental, $P = 0.08$) with increased energy intake. Similarly, when energy was delivered in the form of starch, ruminally or abomasally, acetate incorporation into TNL was elevated or tended to be elevated (mesenteric, $P = 0.02$; omental, $P = 0.06$; subcutaneous, $P = 0.07$) above rates observed for the LI-H_2O animals. For the abdominal adipose depots, the starch effect appeared to be primarily due to the response of the animals receiving abomasally delivered starch, which exhibited numerically higher acetate incorporation rates than their ruminally infused counterparts, although this only achieved significance ($P = 0.05$) for mesenteric adipose, whereas the magnitude of the subcutaneous response to SH infusion was comparable between sites of delivery. Abomasal glucose infusion did not elevate the rate of acetate incorporation into TNL above that observed for the animals infused starch abomasally.

Increased energy intake elevated acetate incorporation into FA (Figure 2) for the subcutaneous tissue ($P = 0.02$), but did not affect omental and mesenteric rates. Acetate incorporation rates were increased in mesenteric ($P = 0.03$) and tended to increase in omental ($P = 0.1$) and subcutaneous ($P = 0.06$) adipose depots with starch infusion. For the abdominal adipose depots, the increase in acetate incorporation into FA was greater for animals infused with starch into the abomasum than for those infused in the rumen. Mesenteric adipose tissue rates of acetate incorporation were lower ($P = 0.03$) for R-SH than A-SH. However, acetate incorporation rate in omental and subcutaneous adipose was not affected by site of SH infusion. Complexity of abomasally infused carbohydrate (A-SH vs. A-G) did not affect incorporation rates.

Increased energy intake tended to increase ($P = 0.06$) glucose incorporation into TNL (Figure 3) for the mesenteric and subcutaneous adipose depots, whereas omental adipose was unaffected by energy intake. Additionally, starch infusion tended to increase mesenteric ($P = 0.06$) and omental ($P = 0.10$) and increased subcutaneous ($P = 0.05$) rates of glucose incorporation into TNL. Rates of glucose incorporation into TNL were not different when site of starch delivery or form of carbohydrate delivered was altered.

Glucose incorporation into FA (Figure 4) was low across treatments and ranged from less than 1 to 9% of the rates generated from acetate incorporation, with the greatest incorporation occurring in omental adipose obtained from steers infused with glucose. Rates of glucose incorporation into FA in the subcutaneous depot

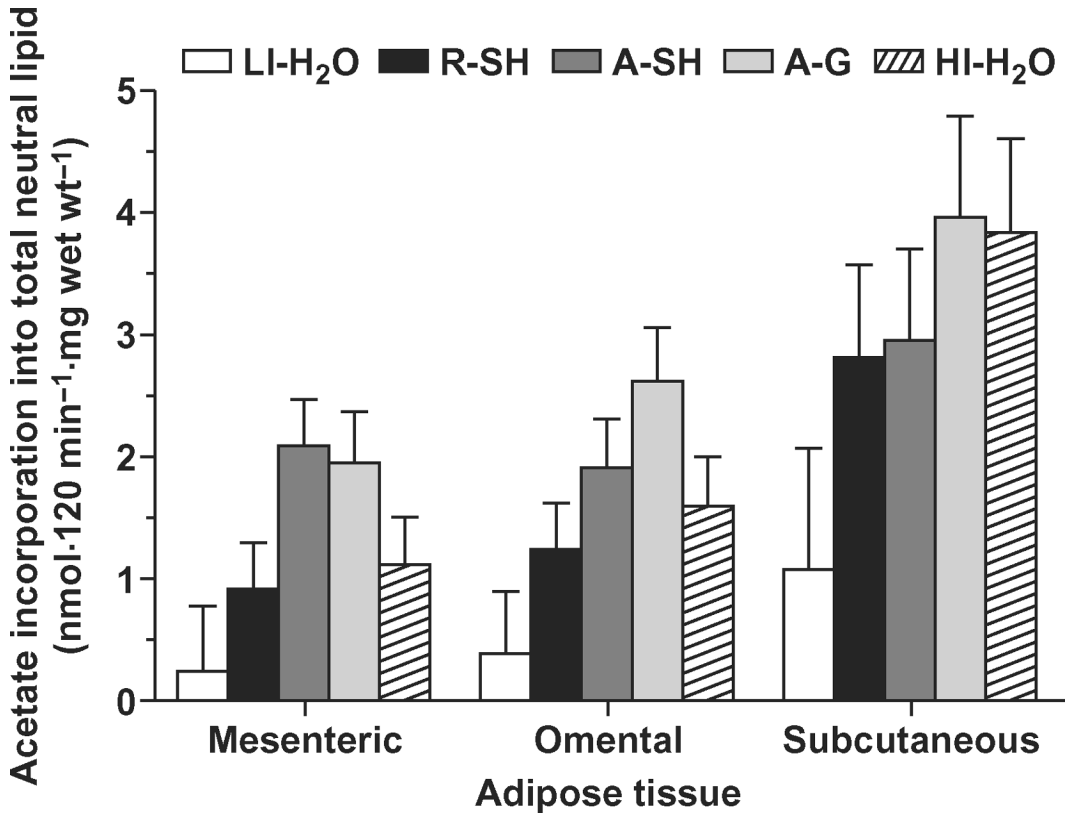

Figure 1. In vitro rates of acetate incorporation into total neutral lipids by adipose tissue isolated from the mesenteric, omental, and subcutaneous depots of steers fed at low intake [LI; 161 kcal of ME/(kg of $BW^{0.75}$·d)] or high intake [HI; 214 kcal of ME/(kg of $BW^{0.75}$·d)] and infused ruminally (R) or abomasally (A) with hydrolyzed starch [SH; 12.6 g/(kg of $BW^{0.75}$·d)] or glucose [G; 14.4 g/(kg of $BW^{0.75}$·d)]. Individual bars represent least squares means with SEM [n = 8, except for LI-H_2O (n = 5) and A-G (n = 7)]. Significant contrasts within adipose site: mesenteric, LI-H_2O vs. HI-H_2O (P = 0.09), LI-H_2O vs. R-SH and A-SH (P = 0.02), and R-SH vs. A-SH (P = 0.05); omental, LI-H_2O vs. HI-H_2O (P = 0.08) and LI-H_2O vs. R-SH and A-SH (P = 0.06); and subcutaneous, LI-H_2O vs. HI-H_2O (P = 0.02) and LI-H_2O vs. R-SH and A-SH (P = 0.07).

were unaffected by dietary intake and starch infusion. However, glucose incorporation rates for mesenteric adipose tended (P = 0.07) to be higher for abomasal compared with ruminal SH infusion. Likewise, compared with abomasal SH, glucose infusion tended to increase (P = 0.08) omental adipose glucose incorporation rates into FA.

Lipolysis, as assessed by NEFA release (Figure 5), was largely unaffected by dietary or infusion treatment. However, rates of glycerol release (Figure 6) were increased (P = 0.04) in subcutaneous adipose and tended to increase (P = 0.08) for mesenteric adipose in steers fed the HI-H_2O compared with the LI-H_2O steers. Starch infusion did not affect glycerol release. Glucose infusion increased (P = 0.008) glycerol release for omental and tended to increase (P = 0.08) release in subcutaneous adipose.

DISCUSSION

Adipose metabolism in the subcutaneous depot has been extensively studied in ruminants as well as other species. Studies of bovine lipid metabolism have been conducted under net lipolytic (lactating cows; Baldwin et al., 1969; McNamara and Hillers, 1986) and lipogenic circumstances (growing beef steers; Smith and Crouse, 1984; Miller et al., 1991; Gilbert et al., 2003). In contrast, few studies in ruminants have specifically addressed metabolism of the mesenteric and omental or abdominal adipose depots relative to subcutaneous adipose. Eguinoa et al. (2003) investigated breed, depot, and age differences in adipose metabolism by evaluating activities of enzymes involved in glycerol 3-phosphate synthesis from glucose (glycerol 3-phosphate dehydrogenase; EC 1.1.1.8), de novo FA synthesis (fatty acid synthetase, **FAS**; EC 2.3.1.85), as well as others, across adipose depots using Holstein and Pirenaican bulls slaughtered at 12 to 14 mo of age. On a cell number basis, Eguinoa et al. (2003) found that the lipogenic activity of the subcutaneous depots was lower than that of the abdominal (omental and perirenal) depots. When corrected for mean cell size, this relationship reversed and subcutaneous activities of glycerol 3-phosphate dehydrogenase and FAS were greater than for abdominal

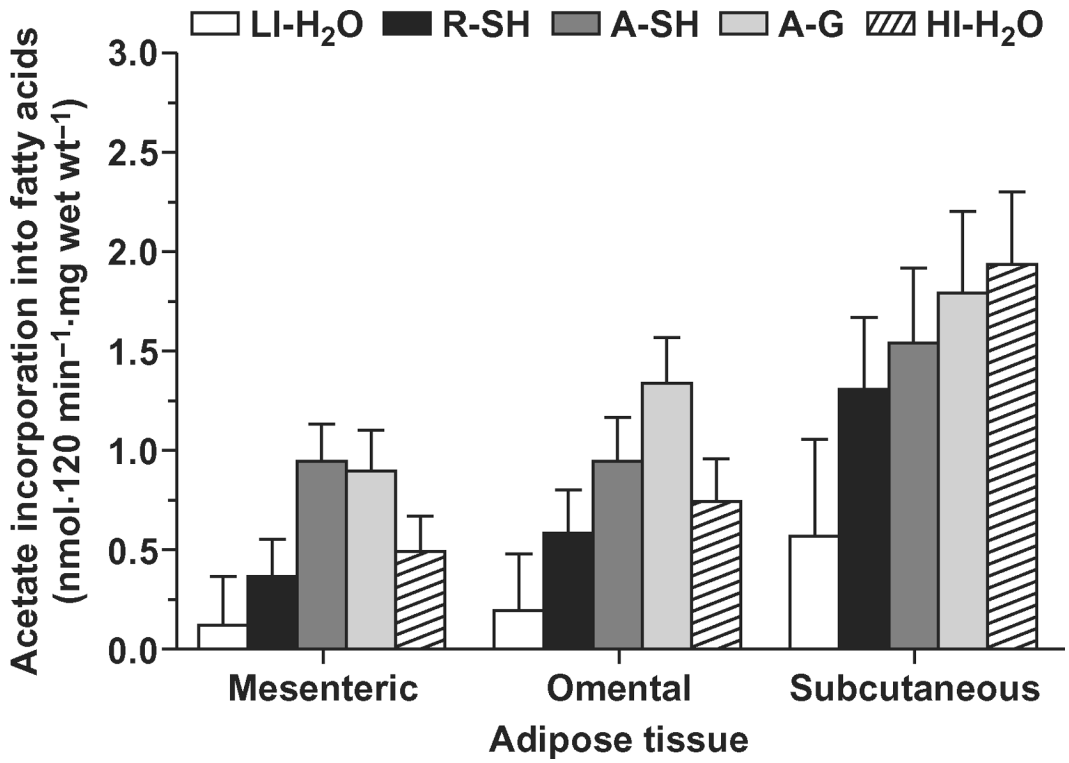

Figure 2. In vitro rates of acetate incorporation into fatty acids by adipose tissue isolated from the mesenteric, omental, and subcutaneous depots of steers fed at low intake [LI; 161 kcal of ME/(kg of $BW^{0.75}$·d)] or high intake [HI; 214 kcal of ME/(kg of $BW^{0.75}$·d)] and infused ruminally (R) or abomasally (A) with hydrolyzed starch [SH; 12.6 g/(kg of $BW^{0.75}$·d)] or glucose [G; 14.4 g/(kg of $BW^{0.75}$·d)]. Individual bars represent least squares means with SEM [n = 8, except for LI-H_2O (n = 5) and A-G (n = 7)]. Significant contrasts within adipose site: mesenteric, LI-H_2O vs. R-SH and A-SH (P = 0.03) and R-SH vs. A-SH (P = 0.03); omental, LI-H_2O vs. R-SH and A-SH (P = 0.10); and subcutaneous, LI-H_2O vs. HI-H_2O (P = 0.02) and LI-H_2O vs. R-SH and A-SH (P = 0.06).

depots (Eguinoa et al., 2003). Similarly, mixed results were observed for acetyl-CoA carboxylase (**ACC**; EC 6.4.1.2) and FAS activity measurements from adipose of wether lambs (6 mo) where similar ACC activities were obtained for subcutaneous and mesenteric depots, but FAS activity was greater (2 times greater) in the mesenteric compared with the subcutaneous depot (Moibi et al., 2000). Using acetate incorporation rates, in adipose isolated from nonlactating ewes (3 to 4 yr) and growing wethers (5 mo), the rate of FA synthesis in the subcutaneous depot was equivalent to that in the omental adipose on a cell number basis (de la Hoz and Vernon, 1993). The current findings extend this observation and demonstrate in ruminants that on a wet tissue weight basis the lipogenic pattern of acetate incorporation into FA and TNL is generally greater for the subcutaneous as compared with the abdominal (omental and mesenteric) depots. Because omental adipocytes typically have greater mean cell volumes than subcutaneous adipocytes (de la Hoz and Vernon, 1993; Eguinoa et al., 2003), comparisons between subcutaneous and abdominal depots of acetate incorporation rates into TNL and FA are both driven toward unity when cell volume is considered (i.e., fewer adipocytes were evaluated for the abdominal depots than for the subcutaneous), which would be consistent with the enzyme activity observations made by Eguinoa et al. (2003) in bovine tissues corrected for cell size. In contrast to the findings with acetate incorporation, rates of glucose incorporation into FA and TNL, with the notable exception of incorporation into FA for omental tissue from the A-G treatment, were largely equivalent across depots. This contrasts data from humans that demonstrated that uptake of glucose by the abdominal adipose is characteristically elevated relative to the subcutaneous depots (Virtanen et al., 2002).

In vivo measures of lipogenesis in cattle have demonstrated that lipogenic rate in subcutaneous adipose is linearly responsive to ME intake (Greathead et al., 2001). However, in vitro studies indicate that lipogenic rate is not only dependent upon ME intake but also energy substrate supply. Pearce and Piperova (1984) evaluated lipogenesis in vitro and enzyme activities in subcutaneous adipose tissue from wethers (9 mo) receiving carbohydrate infusions (dextrin or glucose) via duodenal cannulas and observed increased lipogenic rates with infusion of dextrin relative to noninfused controls when glucose or acetate were provided as substrates in the media. The increase in response to dextrin infusion was greater (2.7 times) when acetate rather

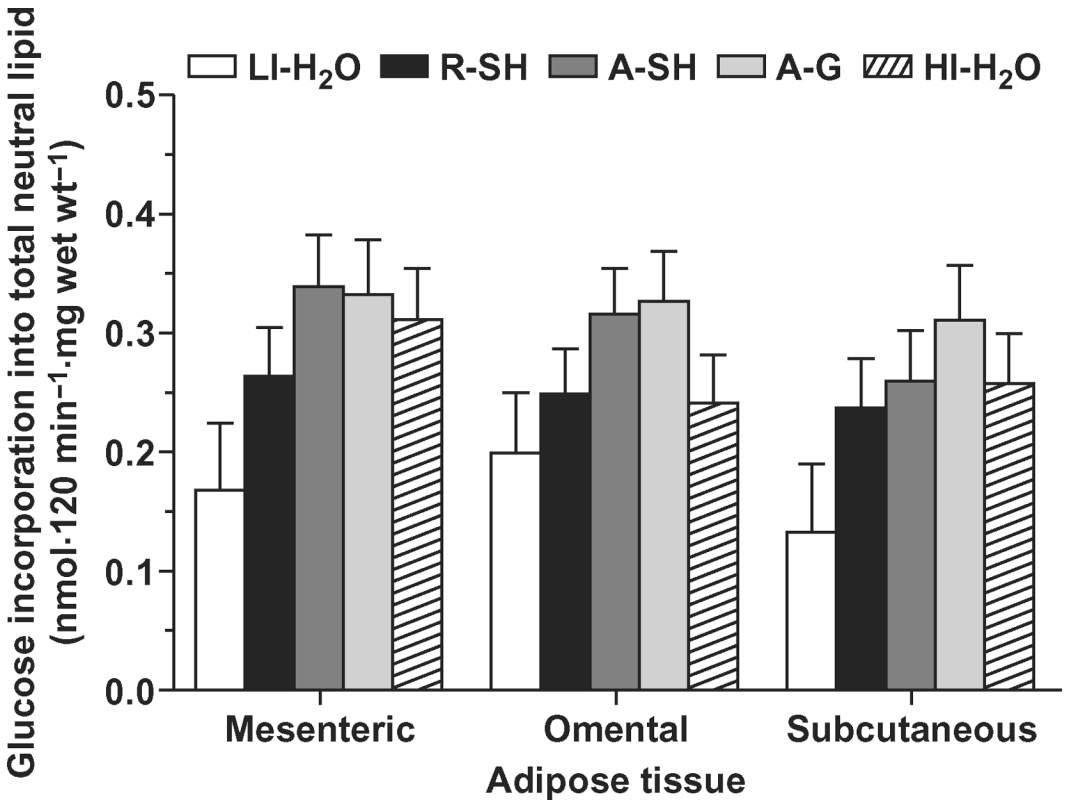

Figure 3. In vitro rates of glucose incorporation into total neutral lipids by adipose tissue isolated from the mesenteric, omental, and subcutaneous depots of steers fed at low intake [LI; 161 kcal of ME/(kg of $BW^{0.75}$·d)] or high intake [HI; 214 kcal of ME/(kg of $BW^{0.75}$·d)] and infused ruminally (R) or abomasally (A) with hydrolyzed starch [SH; 12.6 g/(kg of $BW^{0.75}$·d)] or glucose [G; 14.4 g/(kg of $BW^{0.75}$·d)]. Individual bars represent least squares means with SEM [n = 8, except for LI-H_2O (n = 5) and A-G (n = 7)]. Significant contrasts within adipose site: mesenteric, LI-H_2O vs. HI-H_2O (P = 0.06) and R-SH and A-SH (P = 0.06); omental, R-SH and A-SH (P = 0.10); and subcutaneous, LI-H_2O vs. HI-H_2O (P = 0.06) and R-SH and A-SH (P = 0.05).

than glucose (1.4 times) was used as a precursor. These same researchers also observed a greater increase when glucose rather than dextrin was infused duodenally with rates of acetate and glucose incorporation increasing to 6.7 and 2.2 times more than the control treatment rates, respectively. In the experiments conducted by Pearce and Piperova (1984), ME intake was not controlled and, thus, some of the glucose specific effects could potentially be explained by alterations in intake energy. In our experiment, intake energy was equivalent for the carbohydrate infusion treatments. Thus, these data demonstrate that postruminal supply of carbohydrate increases acetate incorporation into TNL and FA in the abdominal depots, and this is further exacerbated by decreasing complexity of the carbohydrate infused. In fact, acetate incorporation rates in the abdominal depots of the postruminally infused steers were numerically greater than those observed in the HI-H_2O steers, despite lower intake energy. However, this same comparison is less clearly defined for the subcutaneous depot, with increased rates of acetate incorporation into TNL and FA with carbohydrate infusions that were equal to or lower than those in HI-H_2O steers. However, this differential response to substrate supply across depots cannot be definitively ascertained because the actual energy available to the abdominal depots of postruminally infused steers may be greater than those infused ruminally as outlined in the companion paper (McLeod et al., 2007). Regardless, the effects of A-G and A-SH treatments on glucose carbon incorporation into FA across tissues does appear to be sensitive to site and complexity of carbohydrate delivery, and this effect is not evident when only glucose incorporation into TNL is considered.

Early studies in ovine (Ballard et al., 1972) and bovine (Prior and Scott, 1980) adipose tissues established that acetate is used as the primary carbon source for de novo FA synthesis, whereas glucose carbon was incorporated to a much lower extent. However, when carbohydrate availability was increased or when lactate, propionate, or glucose were infused, glucose became a significant contributor to FA in triglycerides in adipose tissue. Prior (1978) further demonstrated that lactate, an intermediate in anaerobic glucose metabolism, was incorporated into carcass fat at 38% of the rate of acetate in ewes fed to achieve positive energy balance. Experiments conducted in vitro with adipose tissue obtained from cattle have shown that glucose, albeit at a

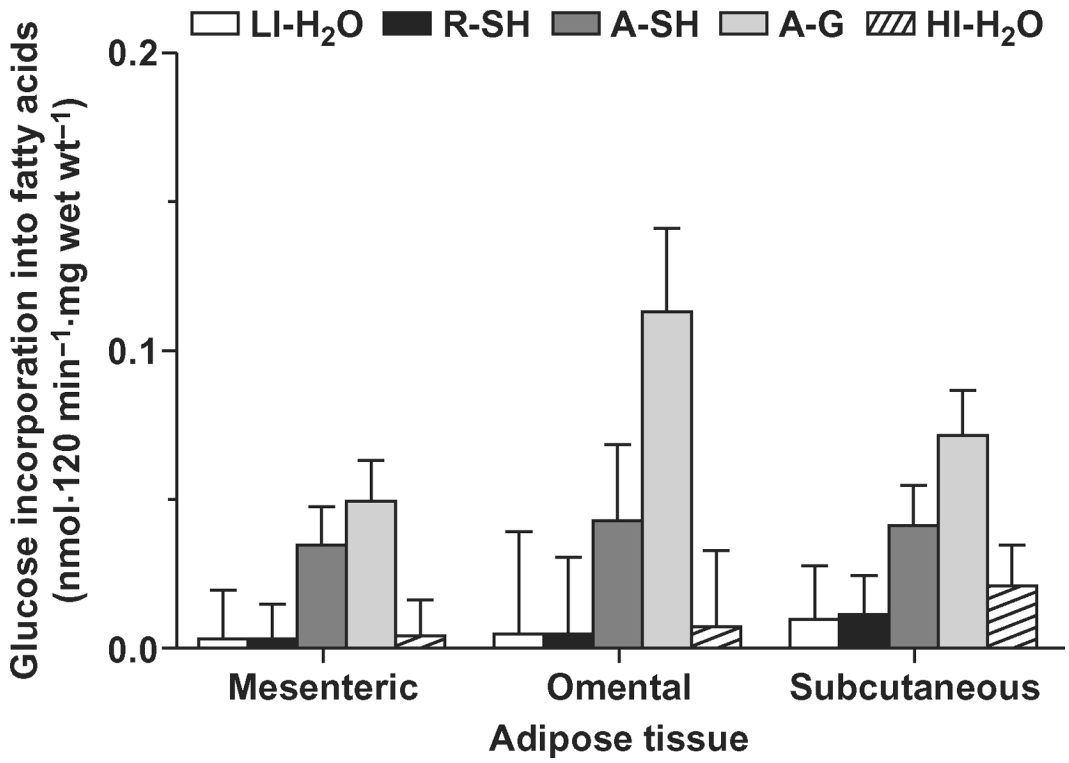

Figure 4. In vitro rates of glucose incorporation into fatty acids by adipose tissue isolated from the mesenteric, omental, and subcutaneous depots of steers fed at low intake [LI; 161 kcal of ME/(kg of $BW^{0.75}$·d)] or high intake [HI; 214 kcal of ME/(kg of $BW^{0.75}$·d)] and infused ruminally (R) or abomasally (A) with hydrolyzed starch [SH; 12.6 g/(kg of $BW^{0.75}$·d)] or glucose [G; 14.4 g/(kg of $BW^{0.75}$·d)]. Individual bars represent least squares means with SEM [n = 8, except for LI-H_2O (n = 5) and A-G (n = 7)]. Significant contrasts within adipose site: mesenteric, R-SH vs. A-SH (P = 0.07); and omental, A-SH vs. A-G (P = 0.08).

much lower rates, is utilized for fat synthesis in alternative fat depots such as the intramuscular adipose (Smith and Crouse, 1984). Indeed, rates of glucose incorporation into TNL in our experiment were substantially lower than rates of acetate incorporation into TNL for all adipose depots examined. Rates of glucose incorporation ranged from a low of 7% (HI-H_2O; subcutaneous adipose) to as high as 70% (LI-H_2O; mesenteric adipose) of rates observed for acetate incorporation for the same tissues. This range is largely a reflection of the extremes observed in acetate incorporations rates across tissues and treatments (i.e., more than 10 times greater acetate incorporation rate between mesenteric adipose in LI-H_2O and subcutaneous adipose in HI-H_2O) combined with the concomitant subtle variations in glucose incorporation rates across the same tissues. A putative control point limiting glucose carbon incorporation into FA is the expression of GLUT4 transporters in the adipose in ruminating cattle (Hocquette et al., 1997). Transcription of GLUT4 is tightly regulated by insulin and glucose in rats (Kahn, 1994) and in restricted and refed cattle expression has been shown to increase in the perirenal depot with refeeding (Bonnet et al., 2004). Using the in vitro approaches employed here, it is not possible to separate changes in transport from changes in metabolism within the adipocyte as the primary limitation to glucose incorporation. Direct measurement of activities of enzymes related to lipogenesis indicates responsiveness to peripheral glucose supply also. Gilbert et al. (2003) found greater FAS activity and NADP-malic dehydrogenase activities in the subcutaneous adipose depot from steers fed rations containing a ruminally protected starch product, which presumably increased small intestinal glucose supply. Moreover, Gilbert et al. (2003) discerned an increase in glucose incorporation into FA, glyceride-glycerol, and TNL fractions above that observed for corn-fed control steers. These responses in glucose carbon incorporation are consistent with cattle refed for 21 d following a 7-d fast where Bonnet et al. (2004) observed increases in lipogenic enzyme activities (FAS, malic enzyme, and glucose-6-phosphate dehydrogenase) in subcutaneous and perirenal depots. In our experiment, ME intake increased or tended to increase glucose incorporation to TNL, but glucose carbon incorporation into FA appears to be dictated by glucose supply. Although statistical significance was only approached for the mesenteric (P = 0.07 for R-SH vs. A-SH) and omental (P = 0.08 for A-SH vs. A-G) adipose depots, the glucose incorporation rates stimulated by abomasal delivery of carbohydrate ranged from 2 to 15 times greater than for ruminal or no infusion (R-SH or HI-H_2O) across depots.

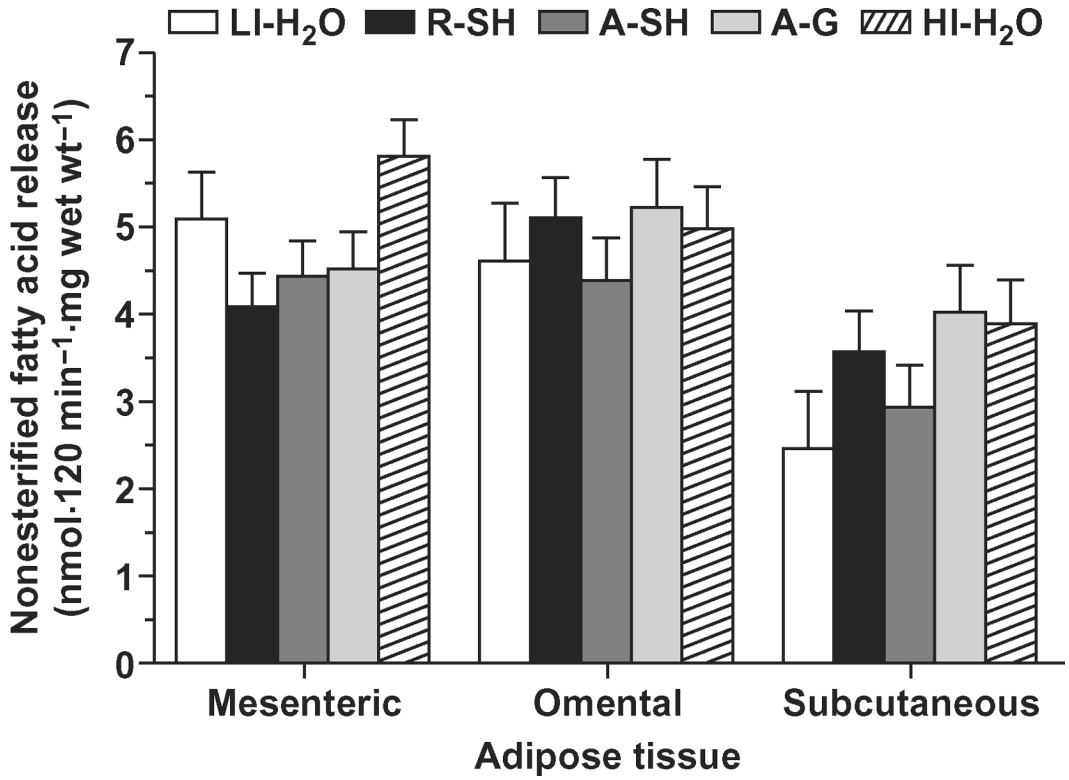

Figure 5. In vitro rates of NEFA release by adipose tissue isolated from the mesenteric, omental, and subcutaneous depots of steers fed at low intake [LI; 161 kcal of ME/(kg of $BW^{0.75}$·d)] or high intake [HI; 214 kcal of ME/(kg of $BW^{0.75}$·d)] and infused ruminally (R) or abomasally (A) with hydrolyzed starch [SH; 12.6 g/(kg of $BW^{0.75}$·d)] or glucose [G; 14.4 g/(kg of $BW^{0.75}$·d)]. Individual bars represent least squares means with SEM [n = 8, except for LI-H_2O (n = 5) and A-G (n = 7)]. Release rate unaffected ($P > 0.10$) by treatment across all 3 adipose depots.

Adipose depot specific substrate preferences for de novo FA synthesis are further supported by the research of Smith and Crouse (1984) who demonstrated that the subcutaneous and perirenal fat depots favored acetate as the primary substrate, whereas intramuscular depots used glucose carbon for lipogenesis. No direct measurements of de novo FA synthesis from glucose were made using intramuscular adipose, but intramuscular fat was unresponsive to dietary treatment or carbohydrate infusion (McLeod et al., 2007), and thus, the effect was nonexistent or too little to detect. Moreover, our data support the observation that FA synthesis from acetate carbon occurs at 10 times the rates of glucose use in the other depots investigated. Further, the capacity of the mesenteric and omental adipose depots also favor acetate to glucose carbon in support of lipogenesis, and this preference is to some degree adaptable based on nutrient supply. Thus, increased adiposity observed by McLeod et al. (2001), using a similar SH infusion model coupled with indirect respiration calorimetery to assess total C and N balance and specific increases in omental, and mesenteric adipose accretion observed in these steers (McLeod et al., 2007) are not simply a function of glucose carbon being diverted toward FA synthesis but rather a result of stimulation of acetate, and to a lesser extent glucose, use for FA synthesis. Observed increases in rate of lipogenesis in the abdominal adipose depots for the postruminal carbohydrate are consistent with mass measurements made in these same steers (McLeod et al., 2007). The mechanism for this stimulation of de novo FA synthesis likely occurs via increased expression of lipogenic enzymes or allosteric effects directly on the metabolic pathway or both. Ballard et al. (1972) suggested that increased reducing equivalents resulting from glucose metabolism as a putative mechanism for increased FA synthetic capacity. However, because the basal media used for the incubations included acetate and glucose, the differential effect of carbohydrate infusion reflects the milieu in vivo and is not a product of limited reducing equivalents. Therefore, the current finding is likely a result of an alternate explanation in this case. Hormonal or metabolite induction or an upregulation of rate limiting enzyme expression could be responsible for this effect. In rat adipose tissue, independent of insulin effects, glucose has been shown to stimulate the expression of FAS and ACC via intracellular glucose-6-phosphate concentration (Girard et al., 1997). Additionally, circulating concentrations of T_3 tended to be increased in A-G steers above concentrations observed in A-SH infused steers. The T_3 has been been shown to increase lipogenic enzymes and regulatory proteins (i.e., FAS, ACC, Spot-

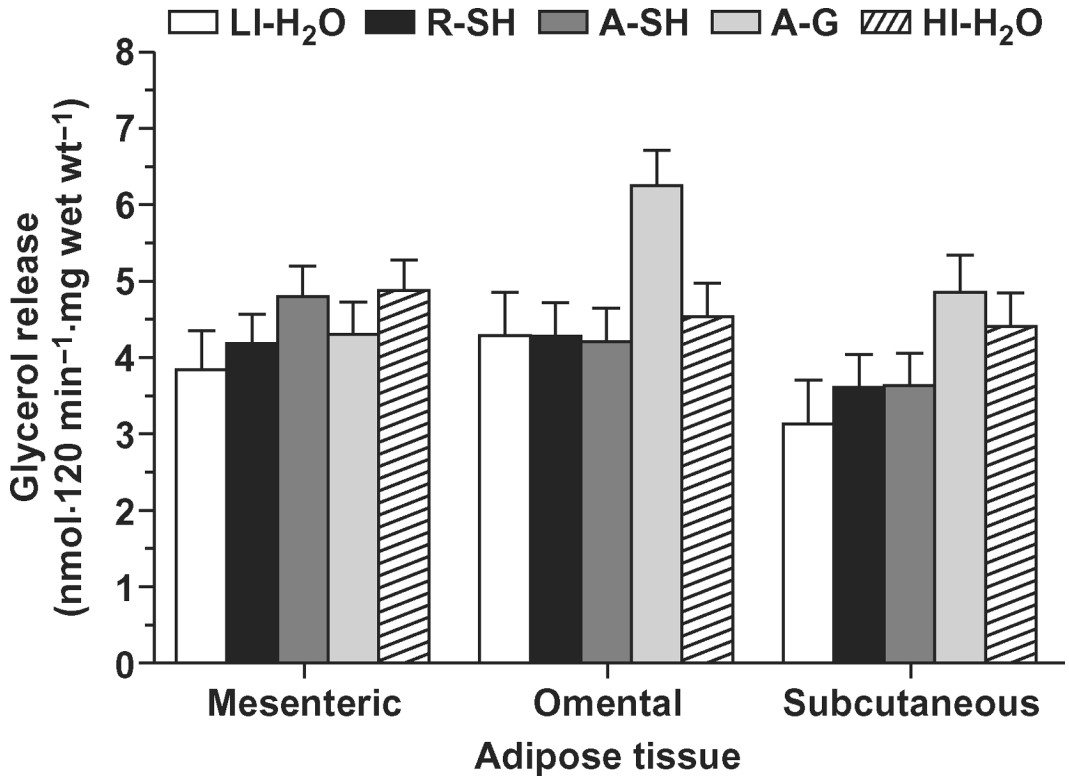

Figure 6. In vitro rates of glycerol release by adipose tissue isolated from the mesenteric, omental, and subcutaneous depots of steers fed at low intake [LI; 161 kcal of ME/(kg of $BW^{0.75}$·d)] or high intake [HI; 214 kcal of ME/(kg of $BW^{0.75}$·d)] and infused ruminally (R) or abomasally (A) with hydrolyzed starch [SH; 12.6 g/(kg of $BW^{0.75}$·d)] or glucose [G; 14.4 g/(kg of $BW^{0.75}$·d)]. Individual bars represent least squares means with SEM [n = 8, except for LI-H_2O (n = 5) and A-G (n = 7)]. Significant contrasts within adipose site: mesenteric, LI-H_2O vs. HI-H_2O (P = 0.08); omental, A-SH vs. A-G, (P = 0.008); and subcutaneous, LI-H_2O vs. HI-H_2O (P = 0.04) and A-SH vs. A-G (P = 0.08).

14). Likewise, IGF-I, which tended to be increased with carbohydrate infusion, has been shown to increase lipogenesis perhaps via increased insulin sensitivity (Etherton and Evock, 1986; Frick et al., 2000; Johansen et al., 2005). The fact that insulin concentrations were similar across treatments does not preclude the possibility that insulin sensitivity may have been greater in tissues from steers receiving postruminal carbohydrate infusions. Moreover, A-G induced increases in the transcription of genes encoding for lipogenic regulatory nuclear proteins carbohydrate response element binding protein, sterol regulatory element-binding protein 1, and Spot 14, as well as their established targets FAS and ACC (Baldwin et al., 2006).

The lack of observed changes in lipolytic rates was not wholly unexpected for our data set because all steers were fed above maintenance. In goats, Dunshea et al. (1990) demonstrated in early lactation that whole body adipose tissue stores were being mobilized to support lactation, yet changes in lipolysis were minor relative to the alteration in lipogenesis. Glucose administration has recently been demonstrated to affect the in vivo NEFA:glycerol ratio of release from human omental adipose through the use of arterio-venous difference experiments. These observations clearly indicate that the short-term regulatory response to glucose administration is through reesterification of NEFA before release resulting in ratios of NEFA:glycerol declining from 3:1 to 1:1 in response to the intravenous administration of glucose. Although baseline or unstimulated lipolysis was not evaluated in our experiment, and thus decreases in baseline lipolytic capacity cannot be completely ruled out as a mechanism responsible for increased abdominal adiposity, the apparent increases in lipogenic capacity with postruminal supply of carbohydrate surpassed the stimulated lipolytic capacity changes observed. Thus, the near 1:1 relationship between NEFA and glycerol release observed in vitro is likely a reflection of a high rate of esterification present in the tissue resulting in reuse of NEFA before release from the adipocyte into the media. Smith et al. (1984) reported that the ratio of FA release to glycerol release declined as steers gained weight and hypothesized that increased reesterification of FA released during lipolysis would account for the decline. Interestingly, mouse and rat estimates of triglyceride synthesis and adipose turnover data obtained in vivo with stable isotopic measurements for glycerol incorporation of ^2H_2O coupled with mass isotopomer distribution analysis revealed higher rates of TG turnover in the mesenteric depot as

compared with subcutaneous depots. Rate of replacement of the nonmesenteric adipose depots were 0.04 to 0.06 vs. 0.21 per d for mesenteric depots translating to half-lives of 12 to 15 vs. 3.3 d for the nonmesenteric and mesenteric adipose depots, respectively. These rates are potentially overestimates of the true NEFA contribution to whole body from the abdominal depots in ruminants, unless reesterification rates are concomitantly evaluated.

In conclusion, current NRC allometric equations are based on the relationship between mature body composition and ME intake and exclude considerations concerning the impact of source of dietary energy supply. Although mechanistic models have been introduced, they are predicated on prediction of protein accretion, with residual energy available for tissue gain assumed to be deposited as fat. In conflict with these assumptions, we have recently demonstrated that the composition of retained tissue energy in growing steers is influenced by not only ME intake, but also by site of carbohydrate digestion and assimilation. In this report we extend those findings by demonstrating that postruminal supply of energy, specifically in the form of carbohydrate, stimulates lipogenesis and this response is more pronounced in the abdominal depots relative to the subcutaneous depot. Additionally, the rate of glucose incorporation into fatty acids is related to glucose supplied.

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